

Some observations on the granivorous feeding behavior preferences of the house mouse (*Mus musculus* L.)

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Abstract

The house mouse (*Mus musculus*) is a highly successful mammal worldwide, in part due to its adaptive consumption of a wide range of seeds, especially those of the agricultural cereal crops. The present study examined the granivorous feeding behavior of mice in relation to wheat kernel texture (hardness), bran color, and the presence/absence of hulls. Three wheat taxa were examined: common bread wheat, spelt, and emmer. There was a particularly strong (5-fold) preference for soft white wheat kernels over hard red kernels. Using near-isogenic wheat lines, the preference was most highly associated with softer texture. This preference was also evidenced in spelt (soft) vs. emmer (very hard) comparisons. For spelt, hulls presented no deterrent to consumption, whereas in emmer, the hulls significantly reduced consumption. In a number of trials, a short lag in consumption patterns of new foods suggested that learning was involved. Regardless of the factor evaluated, all grains were consumed at some level. Soft white wheat was preferred over laboratory pellets by about 4 to 1. The results raise further questions about the ways mice evaluate individual grains and make consumption choices.

Keywords: food preference; grain hardness;
kernel texture; *Mus musculus*; wheat grain.

Introduction

The house mouse (*Mus musculus* L.) is one of the most successful and widespread mammals on earth. It is by nature

an omnivore but has evolved and adapted to be a primary consumer of a wide range of seeds. In large part, the success of the house mouse is due to its adaptation to commensal living with humans (Cucchi et al. 2011). Additionally, its wild/feral populations can adapt to agricultural landscapes, especially those involving annual cereal production. Much of this relation to a small number of cereal grain species is a direct result of the importance of these cereals to *Homo sapiens* L. As further intrigue, the commensal adaptation of the house mouse likely coincided with the establishment of sedentary agriculture in the Fertile Crescent and the cultivation and domestication of cereals (Zohary and Hopf 2000). Clearly, the evolutionary trajectories of mice, humans, and the Fertile Crescent cereal crops are inter-related.

The Food and Agriculture Organization of the United Nations (FAO 2010) estimated world cereal production in 2009 at 2.5×10^9 metric tons (mt), derived from 7.1×10^8 ha, nearly half of the total world arable land. The majority of this grain comes from maize (*Zea mays* L., 7.9×10^8 mt), rice (*Oryza sativa* L., 6.6×10^8 mt), and wheat (*Triticum aestivum* L. and *Triticum turgidum* ssp. *durum* L., 6.1×10^8 mt). Three “ancient” wheats, einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* ssp. *dicoccon* [Schrank] Thell.), and spelt (*Triticum aestivum* ssp. *spelta* [L.] Thell.), are still grown commercially, although their production is very limited (Abdel-Aal and Wood 2005). These ancient wheats are all hulled, and are harvested with non-tenacious lemma and palea. Further, they have semi-tough rachis, which breaks apart during harvest such that the spikelet becomes the morphological dispersal unit of interest (Figure 1).

Rodents demonstrate selective feeding preferences among different seed-based foods. Rats (*Rattus norvegicus* Berkenhout, *Rattus rattus* L.) have been shown in various studies to prefer different tissues or subfractions of grains and seeds (e.g., germ vs. endosperm), meals vs. whole grains, differences due to particle size, and different plant species (e.g., millet vs. maize) (Carlson and Hoelzel 1949, Barnett and Spencer 1953, Khan 1974, Bhardwaj and Khan 1979). Preference differences were also observed among six heteromyid species (Price 1983), and deer mice (*Peromyscus maniculatus* Wagner) (Howard and Cole 1967, Howard et al. 1968, Everett et al. 1978). The common vole (*Microtus arvalis* Pallas) showed no preference for wheat vs. barley, but three *Apodemus* species preferred wheat (Heroldova et al. 2010).

Studies involving the house mouse found preferences among various seed types, meals, and subfractions (Rowe et al. 1974). Pennycuik and Cowan (1990) found that house mice preferred canary seed and maize meal over a commercial diet.

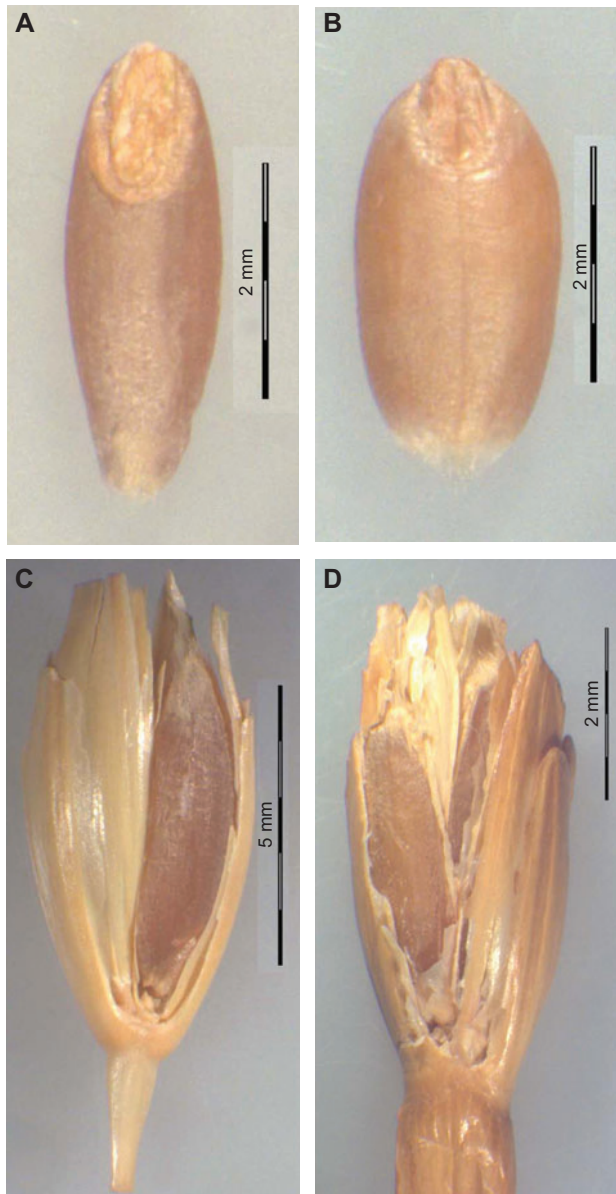


Figure 1 Images of emmer (A) and spelt (B) kernels (caryopses), and intact emmer (C) and spelt (D) spikelets. Scale bars are 2, 2, 5, and 2 mm (A, B, C and D, respectively). In C and D, a portion of the vegetative tissue has been removed to expose the kernel.

Robards and Saunders (1998) found that house mice preference for whole grains depended on the alternatives available. Wheat was preferred over maize, sorghum, rice, and oat, and was similar to canary seed.

Texture appears to be a significant factor in food selection. Soaking maize seeds in water removed all preference of rats (Carlson and Hoelzel 1949), and soaking wheat in water increased consumption (Khan 1974). Increasing hardness of diet pellets decreased consumption by mice (Ford 1977). Robards and Saunders (1998) stated that mice showed a marked preference for grain of soft wheat varieties compared with grain from hard varieties. However, closer examination

of their results indicated that although one variety or grain lot was preferred over others, the preference for soft vs. hard was not absolute. The authors concluded that the preference for soft wheat over hard was maintained even when the grain was extruded with steam to make pellets – a process likely to remove the fundamental “hardness” differences of the two types of wheat.

Other selection factors may be involved. Deer mice find buried seeds by olfactory cues, and odor may play a role in seed palatability (Howard and Cole 1967, Howard et al. 1968). Lawhon and Hafner (1981) reported feeding behavior of kangaroo rats (*Dipodomys* sp.) and pocket mice (*Perognathus* sp.). Each potential food item was handled beneath the head (out of sight), but olfactory cues were apparently not primarily involved; tactile perception was concluded to be the primary means of selecting or rejecting potential food objects. Barnett and Spencer (1953) observed learning behavior; prior exposure had some influence. Rats would often eat the less preferred food. Spencer (1953) described the manner in which mice eat wheat: “Mice typically hold a grain ... at right angles to the long axis of the body, as a man holds corn on the cob ... They also rotate the grain about its long axis while eating it.” Both rat and mouse produced “kibbled” grains where only a portion of the grain was consumed.

Our interest lies in determining how the feeding behavior of the house mouse is influenced by both inter-generic through intra-specific differences among cereal grains, including texture (hardness), morphology, composition, grain color, the presence/absence of hulls (lemma and palea), and ultimately taste and nutrition. In the present report, we examine the feeding preferences of the house mouse in relation to various traits of wheat grains. Our hypothesis is that wheat kernel traits such as hardness, bran color, presence of hulls, and other yet-to-be-identified factors (e.g., smell or taste) influence food selection of the house mouse. The null hypothesis is that none of these factors influence food selection/preference.

Materials and methods

Mice, enclosures, environment

The study covered 144 consecutive days, commencing on January 11, 2010 (day 11), and ending on June 4, 2010 (day 155). Throughout this paper, the dates refer to Julian day (day no.). All animal experiments were approved by the Washington State University Institutional Animal Care and Use Committee (ASAF 03964-001). Female C57Bl/6 mice were randomly selected at 6 weeks of age from a breeding colony originating from The Jackson Laboratory (Bar Harbor, ME, USA; mouse stock number 000664). Mice were provided standard chow and water *ad libitum* and maintained in standard housing (Harlan 70-l paper bedding, Harlan 2018 chow 180 g/kg protein, 14-h light:10-h dark schedule, temperature 20–22°C, 20–30% relative humidity). Mouse cages were (2.8×10⁻³ m length)×(1.7×10⁻³ m width)×(1.1×10⁻³ m height). Cages were filled with

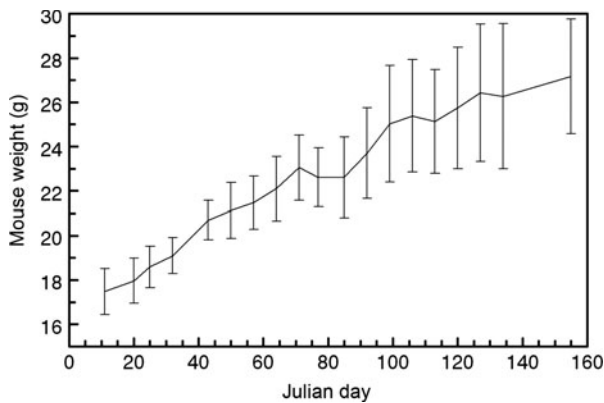


Figure 2 Plot of mouse weights during the course of this experiment. The line represents the mean of 15 mice; vertical bars are standard deviation.

approximately 1.8 l of paper bedding with an average size of 1×10^{-3} m.

Feeders

Initially, stainless-steel cups (28 mm diameter base, 30 mm height, 50 mm top diameter) were used from day 11 through day 41. These were replaced with two-sided plastic feeders (BA330; Penn-Plax, Memphis, TN, USA) (83 mm length \times 76 mm width \times 114 mm height). On day 60, the feeders were modified with a small (ca. 40 mm height \times 35 mm width) piece of uncoated sheet metal that was attached to the feeder with “hot glue.” The sheet metal restricted the grains to the front of the feeder; access was through an opening approximately 28 \times 26 mm (Figure 2). These modified feeders were used throughout the remainder of the study. No trials transversed two feeder arrangements.

The mice were introduced into their boxes about 14 days before the start of the study. “Day” indicates the “end” of a

24-h period. All mice were weighed on days 11, 20, 25, 32, and 43, and then every subsequent 7 days through day 134; a final weight was recorded on day 155.

Wheat grains

The study used exclusively wheat grains (kernels) (Table 1). Louise is a soft white spring and Lassik a hard red spring wheat (*Triticum aestivum*) variety. Four near-isogenic lines (NIL) with contrasting kernel color (white vs. red) and texture (soft vs. hard) were used. They were all derived from backcrosses of the parents early blackhull (EB) hard red winter and Paha soft white winter club wheats (Morris and Allan 2001). In addition, vitreous and non-vitreous kernels were visually selected from the hard red winter EB-Paha NIL grain lot.

In the case of spelt and emmer, de-hulled or “naked” kernels and intact spikelets with kernels *in situ* were also used (Figure 1). spelt and emmer spikelets were donated by René Featherstone (Lentz Spelt Farms, Marlin, WA, USA). Also known as “glume” wheats, spelt and emmer were at times provided to mice as intact spikelets. Spikelets are the harvested, dispersal unit of these hulled wheats wherein the spike disarticulates under moderate mechanical work; however, the kernels remain enclosed in the lemma and palea, i.e., they are not considered “free threshing” like “bread” wheat (*Triticum aestivum* ssp. *aestivum*) (Abdel-Aal and Wood 2005). However, the lemma and palea do not physically adhere to the caryopsis as they do, say, in barley. Kernels were manually removed; in nearly 100% of the spikelets, two kernels are present (this feature was verified in all spikelets provided to mice).

Kernel weight was determined on 50 kernels; kernel texture was determined using the Single Kernel Characterization System 4100 (SKCS) (Perten Instruments, Springfield, IL, USA) (Table 1). The SKCS crushes individual kernels and converts the force-crush profile to a unitless hardness index. Protein (N \times 5.7) was determined by the Dumas combustion method (AACC International 2011) (Approved Method 46-30) (model

Table 1 Wheat grains used in this study.

Identifier	Description	PI number	Kernel weight (mg)	Kernel texture	Kernel protein (g/kg)
Louise	Soft white spring	634865	41 \pm 10	23 \pm 14	111
Lassik	Hard red spring	653535	32 \pm 6	56 \pm 13	134
EB-Paha NIL	Hard red winter	612557	37 \pm 8	54 \pm 15	89
EB-Paha NIL	Soft white winter	612559	28 \pm 5	40 \pm 13	102
EB-Paha NIL	Hard white winter	612558	27 \pm 8	26 \pm 22	95
EB-Paha NIL	Soft red winter	612560	35 \pm 7	65 \pm 16	100
EB-Paha NIL, vitreous ¹	Hard red winter	612557	39 \pm 6	69 \pm 12	99
EB-Paha NIL, non-vitreous ¹	Hard red winter	612557	35 \pm 8	49 \pm 13	75
Spelt	Spikelets and naked kernels	–	38 \pm 8	27 \pm 14	156
Emmer	Spikelets and naked kernels	–	33 \pm 12	79 \pm 12	164

Louise and lassik are commercial wheat varieties; “EB-Paha NIL” denotes near-isogenic lines derived from the hard red winter early blackhull wheat variety and the soft white winter club wheat variety Paha. PI numbers refer to the U.S. Department of Agriculture Agricultural Research Service National Plant Germplasm System accession numbers. Kernel texture is a unitless number obtained from the Perten Instrument’s Single Kernel Characterization System 4100.

¹Vitreous and non-vitreous kernels visually selected from the same grain lot.

FP-528; Leco Corp., St. Joseph, MI, USA) (Table 1). Kernel weight was expressed on an as-is moisture basis; protein on a 140-g/kg moisture basis. The approximate moisture content of the kernels was 75–95 g/kg, which is the equilibrium moisture content of the laboratory environment.

Grains were provided to each mouse in one of three ways: at noon for a 24-h period, at noon for a multiple-day period of up to 4 days (most commonly 3 days over the weekend), or no grain was provided (Table 2). When a grain was provided, it was provided either alone (“independent”) or together with a second grain as a “preference” test (see Tables 2–4). At the end of a 24-h or multiple-day trial, the feeder was emptied of any remaining grain and the entire bedding contents of the box were recovered. New bedding was immediately introduced and the mouse was returned to its box. Most of the time, a new sample of grain(s) was(were) introduced at this time. This entire process required about 4–5 min per box/mouse. Uneaten kernels were recovered from the bedding by sifting, aspiration, and manual sorting. Individual grains were further sorted as described below.

When two wheat grain samples of contrasting bran (testa) color, i.e., “white” vs. “red,” were introduced as a preference trial, the recovered grain in the bedding could be separated by eye. For some grain lots in which the red and white bran colors were not clearly delineated *post facto*, kernels were immersed in an aqueous solution of 1 M NaOH, which enhances the bran color difference (De Pauw and McCaig 1988). In this case, the wet kernels were counted and the average dry kernel weight for that grain lot was used to

calculate consumption. Otherwise, recovered dry grain was weighed directly. Red wheat kernels contain reddish-brown pigments; white wheat kernels contain no pigment (Bradbury et al. 1956).

When two grains of similar color (i.e., white and white, or red and red) were used in a preference test, the grains recovered from the bedding could not be readily identified and sorted according to the original grain lot. Therefore, two marking techniques were examined: the first involved placing a small (ca. 1 mm) dot on each kernel of one grain lot using a fluorescence-based “highlighter” marker (Brite Liner; SociétéBic, Clichy, France). The second involved placing a small (ca. 1 mm) dot on the back of each wheat kernel using “fine point permanent” markers (Sharpie; Sanford L.P, Oak Brook, IL, USA). In this case, all wheat kernels were marked, red on one grain lot and blue on the other. All grains were allowed to set at least overnight before introducing them to the mice to ensure that carrier solvents were completely dissipated. Vitreous and non-vitreous kernels (Morris 2010) were visually sorted from the same grain lot.

Hulled wheats (spelt and emmer) were introduced as either “naked” kernels (i.e., “de-hulled” such that the lemma, palea, glumes, and rachis internode of the spikelet were removed and only the caryopsis was used) or as intact spikelets (Figure 1). In the case of intact spikelets, 50 whole spikelets were provided per mouse per day; the average weight of the 100 kernels associated with 50 spikelets (two kernels per spikelet) was determined for both hulled wheat grain lots (Table 1). At the end of a trial that included a hulled wheat, the uneaten kernels in the bedding and spikelets were recovered, and the

Table 2 Trial and day sequence of house mouse feeding trials over the course of the study, days refer to the Julian day of 2010, the study commenced on day 11.

Trial	Day(s)	Pellets	Wheat grain(s), Comparison	Mice (n)
–	11, 20, 26–27, 33–36, 55, 85, 135–152	+	None	15
1	12, 13, 14, 15, 16–19, 22, 23–25, 30–32, 37–39, 40–41, 42–43, 44–46, 47–48, 51–53, 58–60, 61, 62, 65–67, 72–74, 78–81, 86–88, 89–92, 93–95, 100–102, 107–109, 114–116, 118, 120, 121–123, 128–130, 153, 154, 155	+	Louise, consumption	15
2	21, 117, 119	No	Louise, no pellets	15
3	28, 29	+	Lassik, consumption	15
4	54, 56, 57	+	Louise vs. Lassik	15
5	64	+	Louise vs. hard red EB-Paha NIL	10
6	68, 69, 70, 71	+	Soft white vs. hard red EB-Paha NIL	10
7	75, 76, 77	+	Hard white vs. soft red EB-Paha NILs	10
8	49, 50, 63	+	Louise, unmarked vs. marked with fluorescence	10
9	82, 83, 96, 97, 98, 99	+	Louise, marked with red vs. blue marker	15
10	84	+	Vitreous vs. non-vitreous hard red EB-Paha NIL	7
11	103, 104, 105, 106	+	Spelt naked kernels vs. emmer naked kernels	15
12	110, 112	+	Spelt spikelets (hulled)	15
12	111, 113	+	Spelt naked kernels	15
13	124, 125, 126, 127 ¹	+	Spelt naked kernels vs. spikelets (hulled)	7
14	131, 132, 133, 134	+	Emmer naked kernels vs. spikelets (hulled)	15

For “Day(s),” the Julian days are listed; hyphenation indicates a multi-day trial; commas indicate temporally separated trials. For “Pellets,” “+” indicates that feed pellets were continuously present *ad libitum*; “no” means that no pellets were present during the 24-h trial. The wheat grains are identified in Table 1, “none” indicates that no wheat grain was provided; two-grain preference trials are indicated with “vs.”; kernels marked with a dot of fluorescence or ink are so indicated.

¹Day 127 was eliminated because of errors.

Table 3 Treatment comparisons of the house mouse feeding trials over the course of the study.

Trial no.	Comparison	Trial type	Mice/days	F-values ¹		
				J-Day	Diet	J-Day*Diet
1	Daily consumption of Louise, over time	Independent days	15/75 ²	25.39**	—	—
2	Consumption of Louise with no pellets	Independent days	15/3 ³	1.40ns	41.90**	3.02ns
3	Consumption of Lassik vs. Louise	Independent days	15/2 ⁴	0.06ns	68.49**	12.15*
4	Consumption of Lassik vs. Louise	Preference trial	15/3	26.46**	509.7**	6.79***
5	Louise vs. hard red EB-Paha NIL	Preference trial	10/1	—	3.32ns	—
6	Soft white vs. hard red EB-Paha NIL	Preference trial	10/4	3.44*	984.4**	4.70*
7	Hard white vs. soft red EB-Paha NIL	Preference trial	10/3	0.92ns	12.06*	9.36***
8	Effect of fluorescence marker	Preference trial	10/2, 15/1	12.89**	26.25***	13.04**
9	Louise red vs. blue marker	Preference trial	15/6	1.23ns	0.70ns	2.82*
10	Hard red vitreous vs. non-vitreous EB-Paha NIL	Preference trial	7/1	—	18.87*	—
11	Spelt naked kernels vs. emmer naked kernels	Preference trial	15/4	1.44ns	85.3**	3.35*
12	Spelt spikelets (hulled) vs. naked kernels	Independent days	15/4 ⁵	13.77*	0.00ns	1.45ns
13	Spelt spikelets (hulled) vs. naked kernels	Preference trial	7/3	8.65***	7.05*	0.19ns
14	Emmer spikelets (hulled) vs. naked kernels	Preference trial	15/4	8.74**	17.07*	4.44*

“Independent days” indicate that only one grain was presented to the mice during a 24-h trial; “preference trial” indicates that two different grains were presented to the mice during a 24-h trial. “Mice” is the number of “replicate” mice included in the trial.

¹F-values are significant at *0.05–0.001, **<0.0001, or ***0.001–0.0001.

²Approximately half of these trials were 2–4 days in duration (mostly 3 days over weekends), each was averaged to a 24-h basis; consequently for statistical analysis, 33 observations were used (see Table 2).

³The 3 days of Louise in the absence of pellets were compared with Louise with pellets (days 22, 116, 118, and 120).

⁴The 2 days of Lassik were compared with 2 days of Louise (days 25 and 32).

⁵Two days of spelt spikelets (hulled) were alternated with 2 days of naked spelt kernels.

Table 4 Consumption of grains by house mice as evaluated in various trials.

Trial no.	Grain, trial type	Consumption	Grain	Consumption
2	Louise, no pellets	3.31	Louise, with pellets	2.61
3	Louise soft white, independent ¹	2.67	Lassik hard red	2.09
4	Louise soft white, preference ²	3.00	Lassik hard red	0.61
5	Louise soft white, preference	1.96	Hard red EB-Paha NIL	1.45
6	Soft white EB-Paha NIL, preference	2.66	Hard red EB-Paha NIL	0.56
7	Hard white EB-Paha NIL, preference	See Figure 5	Soft red EB-Paha NIL	See Figure 5
8	Louise, unmarked, preference	See Figure 6	Louise, marked with fluorescence	See Figure 6
9	Louise red marker, preference	ns	Louise blue marker	ns
10	Hard red vitreous EB-Paha NIL, preference	0.94	Non-vitreous hard red EB-Paha NIL	2.11
11	Spelt naked kernels, preference	2.22	Emmer naked kernels	0.68
12	Spelt spikelets (hulled), independent	ns	Spelt naked kernels	ns
13	Spelt spikelets (hulled), preference	2.03	Spelt naked kernels	1.42
14	Emmer spikelets (hulled), preference ³	0.82	Emmer naked kernels	1.55

¹“Independent” indicates that only one grain was presented to the mice during a 24-h trial.

²“Preference” indicates that two different grains were presented to the mice during a 24-h trial.

³See also Figure 7.

kernels were separated from any remaining intact spikelets and included in the consumption calculation.

Data analysis

Occasionally, through some mishap, the data from one or two mice could not be included in the data analysis. Consumption for all trials was calculated on a 24-h, “per day” basis. In all cases, an amount of grain in excess of daily consumption was provided such that some grain was always recovered, and subtracted from the original amount.

All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) of consumption data was performed using “Proc Mixed.” For single-grain consumption (one per day, “independent”) with day-to-day comparisons, the Class statement included “Mouse,” “Day,” and “Diet;” the Model statement included “Day,” “Diet,” and “Day*Diet” interaction. In the dataset, “Day” was coded with sequential numbers such that if grains were provided in the order of, say, A, B, A, B, the consumption data were coded 1, 1, 2, 2. “Mouse” was declared in the “Random” option; the “Repeated” statement set “Subject”

equal to “Mouse” and included the appropriate covariance structure selection [in all cases “ar(1)” see below].

For two-grain preference trials with 2 or more days, the classes were “Mouse,” “Day,” and “Diet.” The Model included “Day,” “Diet,” and “Day*Diet.” “Mouse” and “Mouse*Diet” were declared in the “Random” option; the “Repeated” statement set “Subject” equal to “Mouse*Diet” and included the appropriate covariance structure selection [in trial 7 “un,” in all other cases “ar(1)”].

As a preliminary analysis, ANOVA was conducted with covariance structure set as “Type”=“Compound Symmetry” (cs), “Variance Components” (vc), “Autoregressive” [ar(1)], and “Unstructured” (un) in the “Repeated” statement. In two trials (5, 10) (Tables 2 and 3), a preference trial was conducted for only 1 day. In these cases, the Class statement included “Mouse” and “Diet,” and the Model comprised “Diet” only. “Mouse” was declared Random and no Repeated statement nor covariance structure option was used. Throughout the study, consumption data were calculated, compared, and reported as least squares means.

Results

General growth and observations during the study

The mice were approximately 48 days of age at the start of the study and grew with little apparent rank order change (Figure 2). Beginning and ending mean weights were 17.5 and 27.2 g. Standard deviations indicated that individual mice varied considerably. No mouse died and none were observed to have any health problems.

In general, a considerable amount of wheat grain was removed from the feeders, not eaten, but scattered about the cage. There was no evidence of caching or other purposeful relocation. An “all or none” consumption behavior was primarily observed wherein feeding involved consuming the entire kernel or not at all. There was very little if any evidence

of partially eaten grains (no “kibbling”) (however, see comments on germ consumption below).

Mice preferred eating Louise soft white wheat over commercial feed pellets. During the first 2 days, it appeared that mice rapidly accepted and learned to eat wheat (Figure 3). Consumption was affected by grain availability on days 46, 118, and 120 (see Table 2). Results indicated that the mice preferred a mixed diet and there was a “rebound” effect – a temporary increase in pellet consumption over wheat after a wheat-only day. On day 117, Louise wheat was provided with no pellets. This was the first instance where some mice completely emptied the feeder, even though consumption was about the same (24–36% consumed), the remainder was scattered about in the bedding. This behavior was highly variable among individuals. Owing to the nature of the food pellets and the mice consumption behavior, we determined total daily food intake. However, during the course of the study, there were 3 days during which pellets were withdrawn such that only Louise wheat was available (trial 2) (Table 2). Consumption was highly significantly different (Table 3). In the absence of pellets, mice consumed about 27% more wheat (Table 4). This would suggest that overall, wheat comprised about four-fifths of daily food intake, pellets one-fifth.

Kernel texture and color, *Triticum aestivum*

The first comparison trial involved examining an anecdotal observation that there was a strong preference for soft white

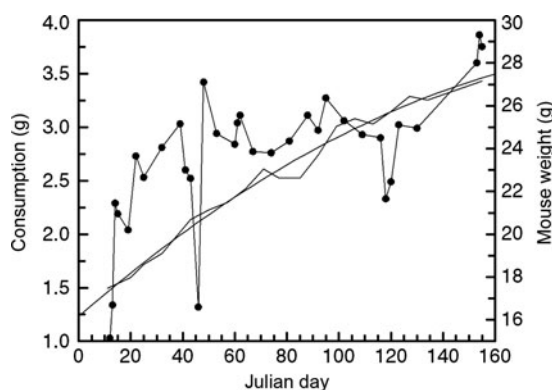


Figure 3 Consumption of Louise soft white wheat grain over the course of the study (line with dots) (trial 1, see Table 1). Values are the mean daily consumption across 15 mice. The two solid lines are mean mouse weights; the smooth line is the second-order polynomial fit.

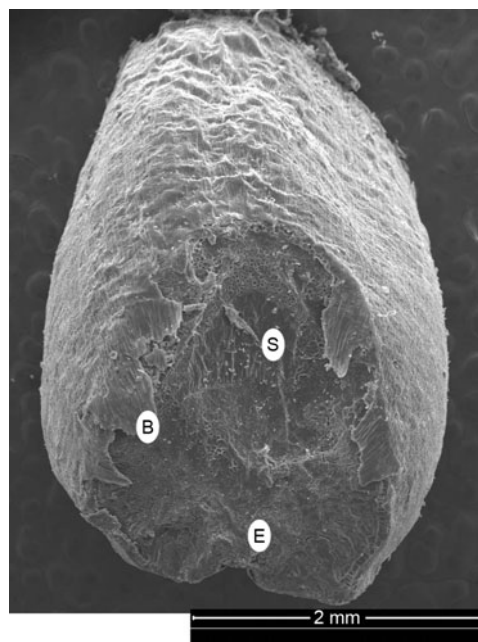


Figure 4 Image showing an example of the selective feeding of the germ from a hard red wheat kernel. Lassik kernel shown. S, remnant scutellum; E, exposed starchy endosperm; B, bran showing exposed edge. The bran that had been covering the germ is gone. Scale bar is 2 mm.

wheat grain over hard red. Using an “independent” day trial (Table 2, trial 3), ANOVA (Table 3) indicated a highly significant difference with Louise soft white being preferred over Lassik hard red (28%) (Table 4). One additional feeding behavior was observed with Lassik and later with EB-Paha hard red NIL, i.e., that mice would consume the germ (more or less), then discard the remainder of the kernel (Figure 4).

The comparison of Louise soft white with Lassik hard red wheat was repeated using the two-grain “preference” system (trial 4, Table 2). In this case where the mice were provided a choice between two wheats, the mice overwhelmingly chose the soft white: means across days were 3.00 g Louise and 0.61 g Lassik ($F=509.7$) (Table 4). This result, a 5-fold preference for soft white over hard red, indicated that kernel texture (i.e., “hardness”), or bran color, flavor, etc., could be the primary discrimination criterion (criteria) for the mice.

To extend this observation, we evaluated a second hard red wheat in a preference trial against Louise (trial 5) (Table 2). Mean consumption was 1.96 g for Louise and 1.45 g for hard red EB-Paha NIL (Table 4). Although this result identified a 35% greater consumption preference for Louise soft white over the hard red, ANOVA did not declare the means significantly different (Table 3). Improvements to the experimental design for such comparisons were considered to involve NIL pairs of grain, all 15 mice, and a greater number of days.

Trial 6 repeated the soft white vs. hard red comparison using two EB-Paha NILs in a preference trial (Table 2). ANOVA produced the largest F-value observed in the entire study (984.4) (Table 3). Again, the preference differential was on the order of 5-fold (4.75) for soft white over hard red (Table 4). This result showed that the mouse preference was a more generalized phenomenon for wheat kernel traits and was not likely due to some variety-specific difference.

In trial 7, we attempted to decouple the kernel texture vs. kernel color traits. In this case, the white wheat NIL had hard kernel texture and the red wheat NIL had soft texture. ANOVA produced moderate levels of significance, the interaction term being largest. A plot of the data (Figure 5) revealed the source of the interaction and an interesting pattern of consumption. On day 75, mean consumption of the hard white vs. soft red

NIL was 1.65 and 1.24 g, respectively (difference not significant). On the following day (day 76), there was a dramatic reversal: hard white consumption dropped to 0.88 g, whereas soft red increased to 2.47 g (significant at $p<0.0001$). This differential preference was somewhat maintained during the next day (day 77) wherein hard white consumption was 1.05 g and soft red was 2.44 g (significant at $p=0.0003$). These results indicated that, perhaps, on the first day, a preference for the white grain had been maintained, but that quickly the preference for soft texture overrode any other consideration. Consumption patterns of individual mice ranged from “very sensitive” to kernel texture (or conversely, potentially, combined with little-to-no sensitivity to bran color) to “little or no preference.”

Marking wheat kernel

Trial 8 addressed the issue of how to identify individual kernels after the completion of a trial when contrasting white vs. red bran could not be exploited. The first method examined was the use of a small dot of fluorescent dye. Attractive features of this marking system were that (1) fluorescence is harmless to mammals (Hara et al. 1998); (2) fluorescence is cheap, readily available, and easy to manipulate; and (3) the use of a small, hand-held LED UV light greatly facilitated the identification of individual kernels during post-trial sorting. Therefore, “marked” kernels were tested in a preference trial (trial 8). A plot of the relative consumption data (Figure 6) showed that on the first day, consumption of marked vs. unmarked kernels was nearly identical. However, on the subsequent day, there was a dramatic reduction in consumption of kernels with a fluorescent mark. This marking procedure was examined approximately 2 weeks later and the large difference in preference was similarly pronounced. These results indicated that although mice did not have a complete aversion to kernels marked with fluorescence, there developed a dramatic negative preference (about 2-fold) and that the behavior may have been learned during the first day of the trial. It was concluded that fluorescent marking was not satisfactory.

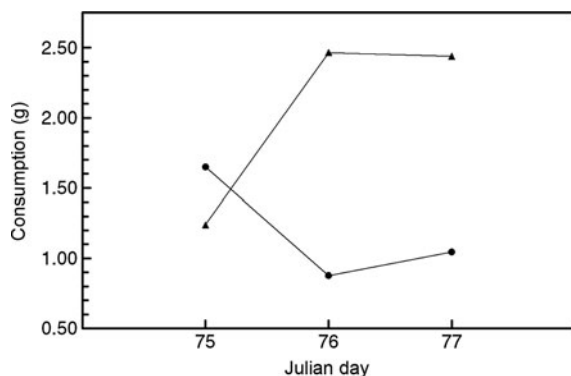


Figure 5 Consumption of EB-Paha hard white (dots) vs. soft red (triangles) NIL wheat grain (trial 7, see Table 1). Mean consumption of all 10 mice.

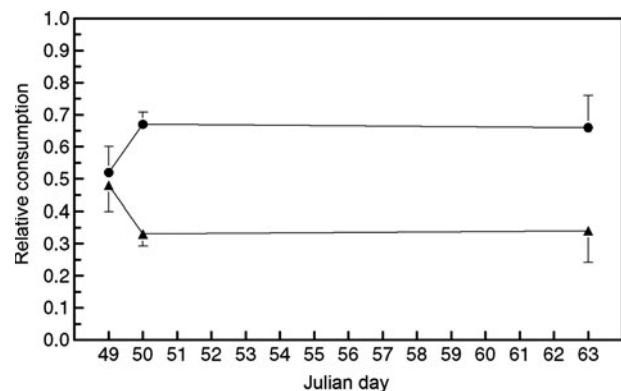


Figure 6 Relative consumption of Louise soft white wheat with (triangles) or without (dots) a small dot of fluorescence on each kernel (trial 8, see Table 1); vertical bars are standard deviation.

Trial 9 evaluated an alternative marking system wherein the effect of a small ink spot made with common consumer markers (“Sharpie”) was examined. To reduce the possibility of a “spot vs. no spot” preference, all kernels were marked. ANOVA returned a marginally significant interaction term ($F=2.82$, $p=0.0019$), and non-significant main effects (red vs. blue, and differences across day) (Table 3). The data for each individual mouse was plotted and examined to see if any mouse had an aversion to one of the two colors or if there was an effect on total consumption. No apparent effect was observed, and along with the ANOVA, the “Sharpie” technique was deemed acceptable.

Vitreous vs. non-vitreous kernels

Both vitreous and non-vitreous kernels were visually sorted from the hard red EB-Paha NIL grain lot and used in a 1-day preference trial (trial 10). ANOVA indicated a significant difference between the two types (Table 3). Non-vitreous kernels were consumed about 2 to 1 over vitreous (Table 4). Whether this result is another manifestation of texture preference or is related to visual perception or some other factor is at present unknown. Non-vitreous kernels were slightly smaller, softer, and had lower protein content (Table 1).

Spelt and emmer wheats

The remaining trials used the more ancient hulled “glume” wheats, spelt and emmer. These two types differ markedly in their kernel texture due to the presence and absence, respectively, of the puroindoline genes (Morris 2002, Bhavé and Morris 2008), and facilitated the examination of the possible effect of hulls on feeding behavior. Trial 11 involved a preference trial using the naked kernels of spelt and emmer (Table 2). The kernels were easily differentiated visually and sorted (Figure 1). Mice preferred spelt over emmer about 3.3-fold (Tables 3 and 4). It should be noted that although spelt is much softer than emmer (Table 1), the kernels are somewhat different in weight and geometry (Figure 1), and that spelt has red bran, whereas emmer has white bran.

Next, we evaluated the effect of the hulls. Trial 12 was set up using 4 alternating days (Table 2) with naked spelt kernels alternating with intact spelt spikelets. This scheme obviated the need to mark kernels, and provided the opportunity to observe feeding behavior when only one grain type was available. The presence of hulls was found to not be a significant deterrent to consumption (Table 4). Again, it may be that greater familiarity with a new food was involved; however, this is bordering on speculation. Loose spelt kernels (removed from the spikelet by the mouse) ranged from 0.0 to 1.14 g/mouse (mean=0.30 g). This activity would seem to represent a waste of energy. Perhaps once the kernel had been removed, the mouse then could make some further assessment as to whether to consume it.

The last two trials examined feeding behavior in “preference” tests (Table 2). Trial 13 used spelt, intact spikelets, and naked kernels. ANOVA identified “Day” as the most significant effect, followed by “Diet” (presence/absence of hulls);

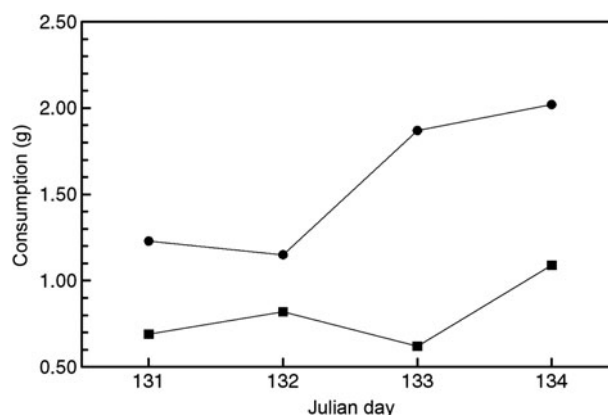


Figure 7 Mean consumption of naked emmer kernels (dots) vs. kernels presented in intact emmer spikelets (squares) (trial 14, see Table 1).

the interaction term was not significant (Table 3). In this trial, mice actually consumed more grain when they had to remove it from the hulls (Table 4). Considering that ANOVA indicated that “Day” was more highly significant than was “Diet,” this result suggested that, at the least, hulls presented no particular barrier to consumption. No particular “learning” trend was evident.

Trial 14 was similar to trial 13, but used emmer. ANOVA indicated that all three model terms were significant (Table 3). Consequently, we examined the basis for the interaction term; the results are presented in Figure 7. Clearly, the ANOVA interaction stemmed from the non-parallel slopes; however, no crossover occurred. Naked kernels were consistently preferred across all days. For intact spikelets, only day 134 consumption differed significantly from day 133 at $p<0.01$ (lower line, Figure 7). In contrast to the results with spelt, these results indicate that the hulls were a significant deterrent and inhibited consumption. Averaged across the 4 days, naked kernels were consumed at a 1.9-fold higher rate (Table 4).

Discussion

Mice are by nature granivores, and in this study they readily consumed a variety of wheat grains that varied in kernel texture (hardness) and bran color. In fact, mice preferred wheat grains over the laboratory feed pellets approximately 4–1. Nevertheless, throughout the study, there was a recurring theme that mice appeared to prefer a “mixed” diet and never chose a single wheat type or food to the complete exclusion of another, regardless of degree of preference. This phenomenon has also been observed in rat (Barnett and Spencer 1953). Why the preference for wheat grains over pellets? Texture might play a role; however, we were not able to test this. Similarly, taste or olfactory cues could play a role. One idea based on the statement of Spencer (1953) might speculatively be that mice prefer to eat foods that can be held in their forepaws. Accessing the pellets required gnawing the pellet

surface between the wires of the cage. Lawhon and Hafner (1981) noted the important possible role of tactile features in food selection in rodents.

More specifically, there was a particularly strong preference for soft white wheat over hard red. This observation was based on two different comparisons: Louise soft white vs. Lassik hard red, and a near-isogenic soft white and hard red pair that would be considered very highly genetically related. In theory, the NILs are, for all intents and purposes, exactly like the original recurrent parent, save the introgressed trait of interest. In attempts to de-couple this texture-color relation, it appeared that, on average, texture was the more important trait. This observation was supported using the EB-Paha NILs, and the spelt (soft and red) vs. emmer (very hard and white) preference trials, and by the vitreous vs. non-vitreous kernels selected from the same grain lot (Tables 1–4). This preference for softer kernels was not slight but ranged on the order of 2- to 5-fold difference. Robards and Saunders (1998) similarly reported house mice preference for soft wheat over hard. However, on closer scrutiny, other factors appeared to be in play, as not all soft and not all hard wheats were equivalent. In this regard, we are interested in possible taste/olfactory preferences among different varieties.

As an extension of texture preference, it may be that the “germ only” consumption (Figure 4) observed with the two hard red grain lots was also related to texture. This point cannot be rigorously examined as we had no means of comparing “hardness” of germ vs. endosperm. Preference by rats and mice for softer texture has been reported, as well as selective eating of the germ (Carlson and Hoelzel 1949, Khan 1974, Ford 1977). Beyond this “selective” feeding, no kibbling was observed (cf. Spencer 1953).

As the mice in our study seem to accept some level of all foods presented to them, the most informative trials were those conducted as “preference” trials, wherein two grains were presented together. In nearly all cases, kernels (and spikelets) were removed from the feeders but not eaten. Therefore, some means of discriminating between two grain lots of otherwise very similar appearance was needed. In this regard, marking kernels with fluorescence was found to be unacceptable, as some feature of the marking system discouraged consumption. Conversely, marking the kernels with a very small ink dot was shown to have no significant negative influence, and facilitated sorting of kernels *post facto*. Twigg and Kay (1992) and Robards and Saunders (1998) found no difference in consumption by mice between dyed and undyed wheat grain. With regard to the presence or absence of hulls, the results were not consistent in that for spelt, the hulls presented no deterrent, whereas in emmer, the presence of hulls consistently reduced consumption. Interestingly, as observed with the spelt spikelets, the vegetative tissues were at times consumed, but more commonly the kernels were removed and eaten. However, some “threshing,” i.e., removal of the kernel without consumption, was observed.

In wild (feral) and commensal situations, the house mouse will generally encounter a number of different food choices including seeds and cereal grains. The present results indicate that on an individual kernel basis, mice selectively exert

feeding preferences, albeit not to the complete exclusion of alternative choices. A clear preference for soft wheat over hard wheat may have implications in the historical commensal context of mouse and man.

In closing, we find it relevant to quote the famous English mouse biologist Peter Crowcroft (1966), drawn from his book, *Mice All Over*, in describing his first experimental mouse subject, “Arthur,”

“There was no particular reason for selecting ... any particular grain of wheat, but Arthur seemed to sniff over a number before selecting one. He was a very well-fed mouse, of course, and he just browsed about, picking up a grain or two wherever he happened to be when he felt like another snack. ... It is tempting to compare Arthur’s behavior with that of a man in similar circumstances. Bananas would be food units of about the right relative size for the experiment. Few men, finding themselves in that improbable predicament, would satisfy their hunger by sitting down and eating bananas from one spot. ... It may be that Arthur was a connoisseur of wheat grains.”

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